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Telomere Length Maintenance And Cardio-Metabolic Disease Prevention Through Exercise Training

Short title: Exercise and Telomeres

Joshua Denham[1], Brendan J. O'Brien[2], Fadi J. Charchar[3,*]

[1]School of Science and Technology, University of New England, Armidale, NSW, Australia.

[2]Faculty of Health, Federation University Australia, University Drive, Mt Helen, Victoria, Australia.

[3]Faculty of Science and Technology, Federation University Australia, University Drive, Mt Helen, Victoria, Australia.

[*]Corresponding author: Fadi Charchar: email: f.charchar@federation.edu.au; phone: +613 5327 6098; fax: +613 5327 9242.

Key points

- Telomere shortening is a hallmark of ageing and is associated with a host of cardio-metabolic diseases prevented or managed by exercise training.
- Exercise training, a superior cardiorespiratory fitness and limited sedentary behaviour may prevent age-related cardio-metabolic disease through telomere length maintenance, though the molecular mechanisms responsible are incompletely understood.
- Future investigations are required to determine the optimal exercise prescription and the regulating factors – including epigenetic modifications, non-coding RNAs, oxidative stress and the telomere position effect – that avoid excessive telomere shortening to prevent premature biological ageing and disease.

Abstract

Telomeres are tandem repeat DNA sequences located at distal ends of chromosomes that protect against genomic DNA degradation and chromosomal instability. Excessive telomere shortening leads to cellular senescence and for this reason telomere length is a marker of biological age. Abnormally short telomeres may culminate in the manifestation of a number of cardio-metabolic diseases. Age-related cardio-metabolic diseases attributable to an inactive lifestyle, such as obesity, type 2 diabetes mellitus and cardiovascular disease, are associated with short leukocyte telomeres.

Exercise training prevents and manages the symptoms of many cardio-metabolic diseases whilst concurrently maintaining telomere length. The positive relationship between exercise training, physical fitness and telomere length raises the possibility of a mediating role of telomeres in chronic disease prevention via exercise. Further elucidation of the underpinning molecular mechanisms of how exercise maintains telomere length should provide crucial information on how physical activity can be best structured to combat the chronic disease epidemic and improve the human health span. Here, we synthesise and discuss the current evidence on the impact of physical activity and cardiorespiratory fitness on telomere dynamics. We provide the molecular mechanisms with a known role in exercise-induced telomere length maintenance and highlight unexplored, alternative pathways ripe for future investigations.

1 Introduction

Telomeres are repetitive DNA sequences located at the ends of chromosomes that function to maintain genome integrity [1]. In most human cells, telomeres shorten with each round of cell division [2, 3] and as such telomere length is reflective of cellular replicative history. Telomeres shorten with ageing and environmental stress, making telomere length a biomarker of biological age. Given the telomere length correlation and synchrony of telomere shortening between somatic cells, leukocyte telomere length is a useful biomarker of telomere lengths from other tissues [4].

Shorter average leukocyte telomere length is typically associated with age-related chronic diseases, such as cardiovascular disease [5-7], type 2 diabetes mellitus (T2DM) [8, 9], some cancers [10], and psychological stress [11, 12]. Furthermore, telomere dysfunction is involved in the pathogenesis of rare genetic diseases, called *telomere syndromes* (reviewed elsewhere [13]). Telomere shortening may also cause the manifestation of non-communicable, age-related diseases. Importantly, the aforementioned diseases associated with short leukocyte telomeres are partly prevented, managed or even regressed by regular physical activity [14-16]. The aim of this review is to discuss and synthesise the current literature on the impact of exercise on telomere biology and discuss potential mechanisms underlying how exercise influences telomere dynamics. Directions for future research will be provided but initially the reader will be introduced to telomere biology and a brief review of leukocyte telomeres and cardio-metabolic disease is provided.

2 Telomere biology

2.1 Telomeres and telomere-associated proteins (shelterin)

Mammalian telomeres are repetitive stretches of non-protein coding DNA (5-TTAGGG-3_n) positioned at the distal ends of linear chromosomes (Figure 1a). Human leukocyte telomere length varies between ~5–15 kilobases (kb) [4, 17] and shortens by approximately 20–50 base pairs (bp) yearly [18]. Telomeres shorten due to inability of DNA replication enzymes to copy the entire 3' end of the leading (G-rich) strand (Figure 1a) [19]. Furthermore, oxidative stress accelerates telomere attrition, with the telomeric DNA GGG triplet most vulnerable to reactive oxygen species [20, 21], and antioxidant compounds attenuate telomere shortening *in vitro* [22]. It is currently unclear precisely how excess inflammation shortens telomeres. It could be that elevated inflammation exacerbates oxidative stress and promotes cellular turnover causing telomere shortening. Once telomere shortening has reached a critical length, telomere dysfunction ensues and signals cellular senescence

through DNA damage pathways – ataxia telangiectasia mutated (ATM), ataxia telangiectasia and rad3 related (ATR), p21 and p53 [23-25]. This, in turn, decreases the proliferative capacity of the tissue leading to an aged phenotype and overall decrease in tissue vitality. There are, however, telomere-associated proteins that repress DNA damage signalling pathways by regulating telomere length; this is primarily accomplished through the formation of unique telomere complexes.

Six telomere-associated proteins (telomere repeat-binding factor 1 [TRF1], telomere repeat binding-factor 2 [TRF2], TRF1-interacting nuclear factor 2 [TINF2], adrenocortical dysplasia homolog [commonly referred to as TPP1], protection of telomeres 1 [POT1] and TRF2-interacting protein [TRF2IP]), collectively known as *shelterin*, bind to the telomeres and regulate their length (reviewed in de Lange [24] and Nandakuma and Cech [26]) (Figure 1b and c). Telomeres and shelterin form unique structures (t- and d-loops [27] [Figure 1d]) and serve as protective caps at the ends of chromosomes to prevent chromosomal end-to-end fusion and allow the complete replication of protein-coding DNA. TRF1 and TRF2 are abundant telomere-binding proteins that play unique roles at the telomeres [28, 29]. While TRF1 negatively regulates telomere length [30], TRF2 protects telomere-mediated chromosomal end-to-end fusion and cellular senescence [31]. TINF2 regulates and interacts with TRF1 and TRF2 [32]. TPP1 and POT1 bind to single stranded telomeric DNA (G-strand overhangs), and interact to recruit and increase the processivity of telomerase resulting in telomere elongation [33, 34]. TRF2 and POT1 independently protect telomeres from DNA damage response pathways (ATM and ATR, respectively) and inevitably prevent cellular senescence through telomere length maintenance [35]. TRF2-interacting protein (TRF2IP) (alternatively known as, RAP1) binds indirectly to telomeric DNA through its interaction with TRF2 and contributes to the prevention of telomere fusion events [36, 37]. RAP1, however, is dispensable as mice lacking a functional *Rap1* gene are viable, unlike mice null for other shelterin components (*Trf1* or *Tpp1*) [38]. RAP1 also has extra-telomeric functions, as RAP1 silences sub-telomeric genes, but also acts as a transcription factor through interactions with other genomic locations [39]. Therefore, shelterin aid in the stabilisation of telomere length, avoids adverse chromosomal events and represses the activation of DNA damage response signalling pathways at telomeres via the formation of t- and d-loops [40, 41]. Although not within the scope of this review, it is important to note telomeres have nucleosomes and histone proteins that are vulnerable to epigenetic modifications [42]. Furthermore, sub-telomeric DNA is heavily methylated and telomere shortening is observed with accompanying epigenetic modifications required for open, transcriptionally active, chromatin [43, 44]. Open telomere chromatin may subsequently up-regulate telomere transcription to produce telomeric repeat-containing RNA (TERRA). TERRA is a long non-coding RNA

molecule that inhibits telomerase by binding to the telomerase RNA template through its complementary and repetitive RNA sequence (5'-UUAGGG-3') [45]. The impact of exercise training on TERRA is currently unknown. Thus, epigenetic modifications and TERRA are added tiers of telomere length regulation. Nonetheless, progressive telomere shortening occurs in the absence of the enzyme *telomerase*.

2.2 Telomerase

Telomerase is a reverse-transcriptase ribonucleoprotein capable of adding telomeric repeats to DNA [46]. In humans, telomerase is made up of two main proteins (telomerase reverse transcriptase [TERT] and telomerase RNA component [TERC]) and additional accessory proteins (dyskeratosis congenita 1 [DKC1] and NOP10 ribonucleoprotein [NOP10]) (Figure 1d). Although telomerase activity is high in human ovaries and testis [47], somatic cells have variable telomerase activity. For example, leukocyte subsets express low to moderate levels of telomerase activity [48], yet telomerase is extremely low or undetectable in human skeletal myocytes [47, 49]. Conversely, telomerase activity is up-regulated in most human cancers and the majority of cancer cells have short telomeres [48, 50, 51]. Remarkably, *in vitro* experiments involving the reintroduction of telomerase to human cells previously lacking telomerase activity extends telomeres and extends cellular life span [3, 52]. Thus, the rate of somatic cell telomere shortening that occurs during ageing is influenced by the proliferative rate of cells, shelterin and telomerase activity.

The regulation of telomerase activity is a complex dynamic process, discussed in detail elsewhere [53, 54]. While POT1 and TPP1 recruit telomerase to telomeres and increase telomerase processivity – the capacity for telomerase to add telomeric DNA [34, 55] – many other non-telomere-associated proteins also govern telomerase activity. Telomerase up-regulatory factors modulated by exercise (acute or chronic) include Akt, insulin-like growth factor 1 (IGF1) and interleukin 6 (IL6), amongst others [53, 56]. Interestingly, accumulating evidence indicates a role for telomerase in the regulation of biological pathways outside that of telomere biology [39, 57].

2.3 Alternative lengthening of telomeres

Alternative lengthening of telomeres (ALT) is another mechanism with the capacity to elongate telomeres independent of telomerase. ALT may occur through telomere sister chromatid exchanges or homologous recombination-dependent DNA replication [58, 59]. Indeed, 10–15% of cancers lack telomerase activity and rely on ALT for telomere length maintenance. Whereas ALT has been reported largely in a relatively small

subset of cancers [60-62], recently ALT activity was observed in normal mouse cells *in vivo* [63]. Common mouse strains, however, have significantly longer telomeres (10 to 80 kb) [64, 65] and the induction of cellular senescence is different compared to humans [66]. Nonetheless, ALT may be a normal part of telomere length regulation and whether exercise training influences telomere length through ALT is currently unknown.

3 Telomeres and cardio-metabolic diseases

Regular physical exercise is not only associated with the decreased risk of developing age-related cardio-metabolic diseases (i.e. obesity, T2DM and heart disease), but exercise training also attenuates and manages particular disease symptoms [67-69]. In this section, we discuss telomere length in context with cardio-metabolic diseases that exercise training is known to prevent or manage. While not discussed here, it is important to emphasise shorter average leukocyte telomeres are found in individuals with elevated psychological stress [11, 12, 70] and those consuming poor diets [71, 72] – lifestyle factors that contribute to an increased risk of many cardio-metabolic diseases.

3.1 Obesity

Most studies on leukocyte telomere length and obesity-related phenotypes – (e.g. body-mass index [BMI], weight and waist circumference) [73-78] – show inverse relationships, though some do not [79, 80]. Childhood obesity has been associated with shorter leukocyte telomeres in both sexes [81], and in boys only [82]. There are reported sex differences, suggesting the adverse effect of obesity-related phenotypes on leukocyte telomere length may be more apparent in women than men [75, 83]. These inconsistencies may be due to the different races and ages of participants studied. Recently, data from weight-loss interventions involving dietary amendments with or without additional physical activity and counselling services support the relationship between leukocyte telomere dynamics and the regulation of obesity-related phenotypes. For instance, increased leukocyte telomere length was correlated with reduced body weight, BMI and waist circumference in middle-aged to older adults (n = 521) after a five-year Mediterranean dietary intervention [74]. A shorter (two-month) intensive lifestyle intervention elicited weight-loss with leukocyte telomere lengthening in adolescents [84]. The reduction in obesity phenotypes caused by the dietary interventions are consistent with the premise that telomere shortening elicits a causal influence on obesity-related phenotypes and that lifestyle strategies aimed at lengthening telomeres may effectively combat disease.

3.2 Type 2 diabetes mellitus

Patients with T2DM have shorter leukocyte telomeres compared to their non-diabetic counterparts [9, 85-88] and the extent of telomere shortening is dependent on patient outcomes. T2DM patients with a history of myocardial infarction (MI) exhibited shorter leukocyte telomeres than those without a history of MI and healthy controls [87]. Additionally, leukocyte telomeres are progressively shorter in T2DM patients with more diabetic complications (such as retinopathy, incipient nephropathy and cardiovascular disease) [86]. The genetic predisposition to elevated oxidative stress could contribute to telomere shortening in T2DM patients. The uncoupling protein 2 (*UCP2*) gene codes a protein important for the electron transport chain and down-regulates reactive oxygen species (ROS) production. T2DM patients with the functional variant allele *UCP2* –866A exhibit shorter (~170 bp) leukocyte telomeres compared to their peers homozygous for the G allele, suggesting a genetic contribution to ROS-induced telomere attrition [9]. Further, leukocyte telomere length shortening over 10 years was related to increases in BMI, insulin, glucose and homeostasis model assessment of insulin resistance [89]. Whereas excessive telomere shortening in T2DM patients is established, whether leukocyte telomere length holds predictive value for T2DM risk is unknown, as two large prospective studies have yielded conflicting results [90, 91].

3.3 Cardiovascular disease

Consistent with T2DM, the presence of cardiovascular disease is characterised by short leukocyte telomeres relative to healthy individuals [92-95]. Accelerated biological ageing is shown in patients with atherosclerosis, such that their leukocyte telomeres are ~300 bp shorter than healthy controls; a difference equating to 8.6 years' worth of telomere shortening [6]. Chronic heart failure patients not only had shorter leukocyte telomeres than age and sex-matched controls, but the extent of telomere shortening was dependent on plaque formation; incrementally shorter telomeres were observed in patients with one, two or three atherosclerotic manifestations, defined as coronary, cerebrovascular and peripheral vasculature, or any combination [96]. Shorter leukocyte telomeres are associated with coronary artery calcification in a cardiovascular disease-free, middle-aged population, suggesting that the leukocyte telomere shortening observed in atherosclerotic diseases may not be a consequence of the disease [7]. The shortened leukocyte telomeres (~300 bp) observed in MI patients indicated patients were biologically 11.3 years older than their age-matched healthy controls [5]. When patients were divided in telomere length quartiles, those with the shortest telomeres had a 3-fold increased risk for MI [5]. Notably, these findings were supported by some [97] but not others [98]. In a large cohort (n >1500), risk of developing coronary heart disease was increased in subjects in the lowest tertile of telomere length and was

attenuated by statin treatment [99]. Whilst the current literature suggests short telomeres are predictive of future coronary heart disease, the predictive ability of telomere length for cerebrovascular disease is unclear [100]. Leukocyte telomere length was not associated with ischemic stroke, in a cohort of 259 males who were initially free from disease that progressed to develop ischemic stroke [101]. The predictive value of leukocyte telomeres in disease prognosis may be limited to middle-aged subjects and not older adults (>75 y) [102], yet older individuals (60 to 97 years) with shorter telomeres have poorer survival due largely to infectious diseases and heart disease [103]. While short baseline telomere length was associated with increased cardiovascular disease-related mortality risk in women, telomere shortening over 2.5-years was associated with greater mortality from cardiovascular disease in men aged 70 to 79 years [104].

3.4 Is telomere shortening a cause or consequence of cardio-metabolic disease?

Considering the available human research, it is important to note that it is unclear as to whether the leukocyte telomere shortening in cardio-metabolic diseases is a cause or consequence of disease. Evidence from mouse and *in vitro* experiments support the former. *Terc*$^{-/-}$ mice, without detectable telomerase activity, exhibit short telomeres and age-related phenotypes – splenic and intestinal atrophy, reduced body weight, haematological abnormalities, reduced proliferative potential, hair loss and tumour formation [105-107]. *Terc*$^{-/-}$ mice from mixed genetic backgrounds also suffer from hypertension [108], depleted haematopoietic progenitor cells [109] and T2DM [110]. Endothelial dysfunction caused by telomere-mediated cellular senescence in *Terc*$^{-/-}$ mice was restored after treatment with antioxidant compounds [111]. Low-grade chronic inflammation and oxidative stress are common symptoms and contribute to the pathogenesis of age-related cardio-metabolic diseases [112-115]. Both inflammation [116] and oxidative stress [21, 117] cause telomere attrition *in vitro*.

Telomere shortening alters the expression of neighbouring – subtelomeric – genes [118, 119] and genes separated by large distances (10 kb) [120], a concept known as the *telomere position effect*. The change in gene expression may not be due to the telomere shortening *per se* but rather a loss of shelterin proteins or telomere chromatin formations [121]. Therefore, telomere shortening may contribute to the manifestation of age-related disease by deregulating transcription of particular genes throughout the genome. Indeed, older versus younger human fibroblasts have shorter telomeres and increased expression of genes involved in chromatin modifying proteins [122], suggesting telomere shortening may facilitate chromatin remodelling through coordinated expression of chromatin modifying genes. Moreover, senescent human fibroblasts with shorter telomeres have differentially expressed sub-telomeric genes compared to quiescent fibroblasts [123].

A genome-wide association study (GWAS) found seven loci containing candidate genes (*TERC*, *TERT*, *NAF1*, *OBFC1* and *RTEL1*) were associated with leukocyte telomere length and risk of multiple cancers, idiopathic pulmonary fibrosis and coronary artery disease in humans, supporting a causal role of telomere shortening in the development of cardio-metabolic diseases [124]. Leukocyte telomere attrition rate over a 7 to 11-year time-period was inversely related to atherosclerosis progression, indicated by carotid intima thickness (cIMT) [125]. Furthermore, subjects with cIMT regression exhibited telomere elongation [125], indicating telomere lengthening is protective of vascular damage. Accordingly, compared to subjects with telomere maintenance or elongation after six years, those with telomere shortening had higher cIMT and an increased risk of cardiovascular events, independent of traditional cardiovascular disease risk factors [126]. Similar associations whereby telomere elongation is observed with attenuated disease symptoms – or vice versa – have been observed in T2DM [89] and obese [74, 84] patients. Although mouse and human studies support the concept that telomere shortening may facilitate disease manifestation and progression, it is important to emphasise other unmeasured factors may mediate telomere and disease dynamics. Lifestyle factors such as physical activity, diet and psychological stress are potential confounding factors that could influence telomere length and disease risk independently. These factors could also facilitate a pro-inflammatory or oxidative cellular environment which could, in turn, promote telomere attrition, cellular senescence and disease.

Importantly, the cardio-metabolic diseases related to telomere shortening are somewhat preventable and managed by physical activity [14-16]. The prevention or management of cardio-metabolic disease conferred by exercise training could be partly driven by telomere length maintenance. Alternatively, exercise training could prevent disease through a reduction in disease-related risk factors (blood pressure, lipid profile, adiposity, etc.), systemic inflammation and oxidative stress, or psychological stress to ultimately prevent telomere shortening.

4 Telomere biology and physical activity

Regular engagement in exercise training is associated with longer telomeres and may attenuate telomere attrition. The optimal exercise recommendations for telomere length maintenance, however, remain elusive. Research has involved the analysis of telomere length in context with physical activity habits, predominantly assessed by physical activity questionnaires, case-control studies involving habitual exercisers and less-active or sedentary controls, and or physical fitness measures (i.e. maximal oxygen consumption [$\dot{V}O_{2max}$] or metabolic equivalent of task [METs]). In the next section, we discuss findings on the effect of physical activity and fitness on leukocyte telomere dynamics mainly in leukocytes and skeletal muscle (Table 1).

4.1 Telomere length and physical activity

Physical activity was positively correlated to leisure-time physical activity levels in 2401 twin volunteers [127]. The leukocyte telomeres were 200 bp longer in individuals who performed the most physical activity compared to those who engaged in the least amount [127]. Moreover, in twins discordant for physical activity levels, leukocyte telomeres were 80 bp longer in the twin engaging in the most physical activity [127]. Supporting these results, a linear relationship between physical activity and telomere length was observed in a cohort of female nurses [128], suggesting that physical activity may be protective of telomere length.

There may be a threshold for the amount of physical activity ideal for telomere length maintenance. An inverted-U relationship was observed when 69 healthy subjects were divided into estimated energy expenditure quartiles, with those expending moderate amounts of energy exhibiting the longest peripheral blood mononuclear cell (PBMC) telomeres [129]. Strikingly, longer leukocyte telomeres and a lower percentage of short telomeres were found in men who engaged in moderate physical activity compared to men who engaged in low or high amounts of activity 29 years prior to telomere length assessment [130]. Despite supporting an inverted-U relationship between physical activity and telomere length [129], physical activity was assessed from self-reported questionnaires [130] which often do not yield valid or reliable results. There are also some studies that do not show any statistically significant associations between physical activity and leukocyte telomere length [131-133]. Interestingly, recreational physical activity may be more important for telomere maintenance as job-related physical activity was not associated with leukocyte telomere length in 981 individuals (aged 45–84 y) [134]. Physical fitness, however, was not measured in these studies and the lack of association between telomere length and physical activity may be partially explained by the physical activity assessment methods used.

Habitual or regular exercise training seems to protect against telomere shortening, particularly in postmenopausal women. In cross-sectional analyses, postmenopausal women who performed 60 min of resistance and aerobic exercise more than three times per week for an average of 19 months exhibited significantly longer PBMC telomeres compared to their sedentary peers [135]. Postmenopausal women with stage I-III breast cancer, who participated in moderate to vigorous physical activity also have significantly longer PBMC telomere length compared to their sedentary counterparts [136], suggesting exercise intensity may be an important determinant of telomere length. Conversely, recent data indicates that sedentary behaviour is detrimental to leukocyte telomere length. In a large epidemiological study, screen-based sedentary behaviour

was inversely associated with leukocyte telomere length in 6405 adults (20 – 84 y) [137]. Consistent with these findings, telomere lengthening was observed in older adults who reduced their sitting time, after a six-month intervention involving prescribed exercise and counselling [138].

4.2 The impact of age on telomere length and physical activity

The positive effects of physical activity on telomere maintenance may be restricted to certain age groups. In older adults (≥65 y, n = 2006) physical activity assessed by the Physical Activity Scale for the Elderly [139], was not associated with leukocyte telomere length. Rather than physical activity, physical capacity may have a greater impact on telomere length maintenance in older adults. For example, gait speed [140] and Barthel index [141, 142] – an assessment of ability to perform activities of daily living, were positively correlated to telomere length in older adult populations. In a cohort of 548 same-sex Danish twins, leukocyte telomere length increased with every unit increase in physical ability score [143]. Moreover, a 10 year longitudinal study indicated a decline in grip strength was related to a faster leukocyte telomere attrition, potentially caused by elevated circulating inflammatory markers (interleukin-1β and cortisol) [144]. Sit to stand performance and walking distance were associated with longer leukocyte telomeres in a cross-sectional analysis involving 582 older adults, while estimated energy expenditure and time to complete five chair stands were associated with attenuated telomere attrition over a 5-year period, suggesting physical activity and performance are important for telomere maintenance even in later years of life [145].

Duration of physical activity may not be as important as the intensity of exercise for younger individuals. One study involving 667 adolescent subjects revealed physical activity, objectively assessed by accelerometers worn for seven days, was unrelated to leukocyte telomere length [146]. Telomere length was, however, positively associated with exercise intensity [146], providing evidence that vigorous exercise may be protective of telomeres in young individuals.

4.3 Endurance exercise, cardiorespiratory fitness and telomere length

Endurance athletes have a superior level of cardiorespiratory fitness, resulting from prodigious amounts of aerobic exercise training. They also have a much lower risk of cardiovascular and cancer related mortality, and live longer than sedentary individuals [147]. Interestingly, telomere attrition was attenuated in middle-aged (~51 y) German track and field athletes who ran an average of 80 km a week, such that they had similar PBMC telomere lengths compared to younger (~21 y) athletes and sedentary controls [148]; findings that have been

supported by others [149]. Ultra-endurance athletes running an average of 40–100 km per week possessed 11% longer leukocyte telomeres compared to healthy subjects, indicating these athletes had prevented approximately 16 years' worth of age-related telomere shortening [150]. Of note, we recently verified our previous findings that endurance athletes have longer leukocyte telomeres and attenuated biological ageing [150] in another cohort of endurance (triathletes, long distance runners and cyclists) subjects, such that the athletes possessed longer telomeres than controls and had prevented 10.4 years of telomere shortening caused by ageing [151]. In a much smaller (athletes n = 17, controls n = 15) study, however, athletes running ~33 km a week had similar granulocyte and lymphocyte telomeres to that of sedentary controls [152]. The discrepancy maybe due to the leukocyte subsets analysed or method of telomere measurement – quantitative fluorescent in situ hybridization (qFISH). Therefore, the majority of available literature finds strenuous, endurance exercise training is associated with longer telomeres. The adherence to regular exercise training may be required in order to prevent telomere shortening as former Finnish elite athletes possessed comparable leukocyte telomere lengths to that of their sedentary counterparts [153]. It is currently unclear as to why telomere length seems to be somewhat maintained in these athletes, but there is mounting evidence indicating a role for cardiorespiratory fitness.

Maximal oxygen uptake was an independent predictor of leukocyte telomere length in a cohort of 57 young (21 y) and older (~62 y) adults, and explained 20% of the overall variance in leukocyte telomere length [149]. Others have also demonstrated weak to moderate (r = 0.11 to 0.44) positive correlations between leukocyte telomere length and VO_{2max} [149, 154]. Leukocyte telomere length was ~169 bp longer in stable chronic heart failure patients with high (> 7 METs) fitness compared to their unfit (< 5 METs) peers [155]. In a large epidemiological study including 1764 US adults, predicted maximal oxygen uptake was associated with longer telomeres, such that individuals with moderate (39.1 $ml \cdot kg^{-1} \cdot min^{-1}$) or high (50.9 $ml \cdot kg^{-1} \cdot min^{-1}$) cardiorespiratory fitness possessed longer leukocyte telomeres than those with poor fitness (30.7 $ml \cdot kg^{-1} \cdot min^{-1}$). The positive relationship between VO_{2max} and telomere length is not exclusive to leukocytes, as skeletal muscle telomeres are longer in subjects with higher VO_{2max} [156]. Thus, improvement in cardio-respiratory fitness seems an important adaptation potentially involved in telomere length maintenance caused by exercise training.

It is important to emphasise that while ample evidence indicates that endurance exercise is associated with longer leukocyte telomeres in athletes compared to less-active controls, data on skeletal muscle telomeres is not as consistent. Unlike highly proliferative leukocytes, skeletal muscle telomeres are quiescent and the limited available data demonstrate recreational physical activity does not cause excessive telomere shortening in young

or older adults [157]. Skeletal muscle telomeres may not succumb to chronological ageing. Conversely, the increased oxidative stress associated with ageing may facilitate telomere shortening, as older mobile adults have reduced reactive oxygen species and longer leukocyte and thigh skeletal muscle telomeres compared to their immobile peers [158]. Endurance athletes, however, with exercise-associated fatigue possess short muscle telomeres [159]. Healthy athletes have been documented to have longer [156] or comparable [160] muscle telomere lengths relative to sedentary controls. Resistance exercise-trained athletes have borderline significantly longer minimum and mean telomeres compared to controls [161]. Therefore, current data on endurance exercise and resistance training suggest that endurance exercise is not detrimental to leukocyte or muscle telomere length maintenance, though there could be an upper limit to the protective effect of exercise, considering shorter muscle telomeres were inversely correlated to maximal strength [161] and training history (years and hours spent running) [160].

4.4 Longitudinal, prospective studies on lifestyle changes and leukocyte telomere length

Well-controlled lifestyle interventions are beginning to provide insights on the impact of exercise training on the age-associated telomere attrition. A three month intervention consisting of a healthy diet (high in natural food products), stress management, social support and 30 minutes of walking exercise six days a week significantly increased PBMC telomerase activity in low-risk prostate cancer patients, who refused traditional cancer treatment [162]. After a 5-year follow-up, the experimental group had modestly longer telomeres, an effect mostly unobserved in the controls [163]. Whilst these preliminary findings propose a healthy lifestyle may increase telomerase activity and telomere length the study was small (cases n = 10, controls n = 25) and some cases in the lifestyle intervention experienced telomere shortening comparable to the controls. Similar findings were reported in a group of 59 middle-aged sedentary males after a much shorter (6-month) moderate-intensity exercise training intervention [164], although a control group was not included. In a large, 12-month randomised controlled trial, women in either the control (n = 87), exercise-only (n = 117), calorie-reduced diet only or exercise (n = 117) and calorie-reduced diet (n = 118) groups showed a similar, minimal change in leukocyte telomere length that was not statistically significant [154]. Therefore, it is premature to claim whether exercise training can solely prevent telomere shortening or induce telomere elongation. Additional controlled exercise training studies involving different exercise prescription will be required to identify whether exercise alone can prevent age-associated telomere shortening.

Evidence indicates psychological stress is detrimental to telomere length maintenance [11, 12]. Importantly, the negative association between psychological stress and leukocyte telomere length was abolished in postmenopausal women achieving the Center for Disease Control and Prevention's recommended physical activity levels (75 min of vigorous exercise per week) [165]. Strikingly, over a one year follow-up period, postmenopausal women who experienced more adverse life stressors experienced greater telomere attrition (~35 bp decrease for every life stressor), however telomere attrition was attenuated by a healthy lifestyle (more physical activity, better sleep quality and a healthier diet) [12]. When telomere attrition and physical activity levels were analysed independently, a one standard deviation below or at the group average was associated with a significantly faster telomere attrition [12]. Thus, exercise training may combat psychological stress-induced telomere shortening.

Taken together, physical activity seems to confer a beneficial effect on leukocyte telomere length maintenance as shown by association studies. Despite positive correlations between physical activity levels and leukocyte telomere length, the ideal exercise prescription for telomere conservation is not yet established. Endurance athletic status is associated with longer leukocyte telomeres predominantly in middle-aged subjects. No evidence indicates extensive aerobic exercise is associated with shorter telomeres in healthy subjects. The inconsistencies throughout the literature are likely due to genetic diversity of subjects, age ranges, DNA extraction methods [166], methods of assessing physical activity and telomere length quantitation [167-169], and the controlling of other confounding factors (eg. diet, psychological stress and socioeconomic status). Researchers should control for the aforementioned confounding factors to attempt to isolate the impact exercise training has on telomere biology. Notably, it is unclear as to whether individuals who inherited and were born with long telomeres have increased cardiorespiratory fitness and consequently engage in more exercise training. Indeed, there is a genetic propensity for $\dot{V}O_{2max}$ [170] and the training-induced responsiveness to $\dot{V}O_{2max}$ [171], making it plausible that physical activity data may be purely associational and that genetic influence programs telomere maintenance. It is suggested that literature on telomere length and physical activity assessed solely by self-reported physical activity questionnaires should be interpreted with caution as they have inherent issues such as recall-bias and over/under-estimation of actual physical activity levels is common [172-174]. Whereas the benefits of aerobic exercise on telomere length are commonly reported, data on the effects of resistance training on telomere length are scarce. It is recommended future research should consider quantifying cardiorespiratory fitness in context with self-reported physical activity and telomere dynamics. Additional randomised, controlled exercise training interventions and longitudinal telomere length assessment with healthy

and clinical populations will help discern the involvement of telomere regulation on disease management and regression. Further research is also required to elucidate the ideal exercise prescription required for telomere length maintenance in other unexplored tissues. This knowledge in turn can be used to optimise the physical training required to combat cellular ageing and prevent age-related cardio-metabolic disease.

5 Molecular mechanisms in exercise-induced telomere dynamics

5.1 Telomerase activity

Physical activity and exercise training are associated with longer telomeres but the molecular mechanisms underpinning the relationship are not very well understood. The ability of telomerase to extend telomeres and prevent telomere shortening-induced cellular senescence [3, 46] suggests a crucial role for increased telomerase activity mediating telomere length consequential to exercise training. Indeed, elevated telomerase activity after chronic exercise training has been demonstrated in a variety of tissues, including human PBMCs [162, 164], mouse skeletal muscle [175], heart [176], aorta [148] and hippocampi [177]. Endurance athletes who regularly engage in rigorous aerobic exercise exhibit a 2.5- and 1.8-fold increased telomerase activity compared to young and middle-aged sedentary subjects, respectively [148]. Despite the capacity of chronic exercise training to influence telomerase activity, whether an acute bout of exercise has a similar effect remains to be explored. The exercise training-induced increase in telomerase activity is dependent on TERT and endothelial nitric oxide synthase (eNOS) protein expression, as long-term exercise training does not modulate telomerase activity in *Tert*$^{-/-}$ or *eNos*$^{-/-}$ mice [176]. Mice treated with recombinant growth hormone or IGF1 exhibited an 8- and 14-fold increase in cardiac telomerase activity, indicating these growth factors are mediators for the up-regulation telomerase activity in cardiac myocytes of exercised mice [176]. These data provided crucial evidence elucidating a role for growth hormone, IGF1, eNOS and TERT in the up-regulation of telomerase activity induced by exercise training.

5.2 Shelterin expression

Telomerase must be recruited to telomeres for telomere elongation to occur; a process tightly regulated by shelterin proteins [26, 54]. 30 minutes of aerobic exercise at 80% of peak oxygen consumption (VO_{2peak}) significantly up-regulates leukocyte *TRF2IP* and *TERT* expression in healthy young men [178]. Whereas acute aerobic exercise modulates some telomere-associated genes in leukocytes, this may not affect protein abundance. For example, PBMC *TRF1*, *TRF2* and *POT1* expression were all up-regulated the day following a

seven-day ultra-marathon race in eight athletes, but this did not translate into increased protein abundance or telomerase activity [179]. MicroRNAs (miRNAs) are small RNA molecules that negatively regulate translation or degrade messenger RNA (mRNA) targets, in turn, limiting protein abundance. The acute exercise-induced decrease in expression of the miR-96 and -186-targeted transcript *TERF2IP* was observed with a concomitant increase in miRNA molecules [178]. miRNA regulation of shelterin genes is a possible explanation for the unchanged protein abundance observed previously [179]. Whilst seven-days of intense endurance exercise may not facilitate shelterin protein abundance, the overall adaptation to endurance exercise may lead to altered shelterin protein expression. 57 endurance-trained athletes showed increased PBMC *TF2* mRNA and protein levels, with increased telomerase and longer telomeres compared to sedentary subjects [148]. Similarly, aortic [148] and heart [176] *TRF2* mRNA and protein levels are increased in mice after three weeks of voluntary wheel running. Therefore, some shelterin proteins particularly TRF2, are modulated by long-term exercise training in PBMCs, heart and aortic tissue. There is, however, limited evidence showing the acute effects of exercise on shelterin expression.

Relative to other tissues, skeletal muscle shelterin may not be as responsive to exercise training. In athletes, no changes were observed in skeletal muscle *TRF1*, *TRF2* or *POT1* expression after a seven-day ultra-marathon [179]. However, the age-related increase in TRF1 protein expression in plantaris muscle of sedentary mice was attenuated in exercised mice [175], possibly through p38 mitogen activated protein kinase (MAPK) inactivation [180]. Considering TRF1 is a negative regulator of telomerase activity [54], the decreased TRF1 expression observed in conjunction with increased telomerase activity [175] may be a mechanism to counteract the telomere shortening observed in the exercised mice. TRF1 protein expression is decreased after acute exercise in mouse skeletal muscle [180], but whether this is detrimental or occurs in conjunction with increased telomerase activity is currently unknown.

5.3 Other mechanisms

Exercise training prevents many age-related metabolic diseases including cardiovascular disease [181], T2DM [182] and obesity [183], and is reflected by a longer telomere phenotype. Conversely, age-related chronic diseases are associated with shorter mean leukocyte telomere length [184] and are all characterised by low-grade chronic inflammation and oxidative stress [185]. Cell culture experiments indicate the former accelerates telomere shortening [116]. Oxidative stress also induces telomere attrition [22, 186], with the telomere GGG sequence particularly vulnerable to damage caused by reactive oxygen species [21]. Importantly, regular

exercise training reduces both low-grade chronic inflammation and circulating oxidative stress [187, 188]. We, however, found no correlations between long-term exercise training, telomere length and inflammatory cytokines (interleukin-6, intercellular adhesion molecule-1 e-selectin, C-reactive protein and leptin) [150]. Additionally, a six month exercise training intervention in obese subjects improved antioxidant enzyme activity but did not alter leukocyte telomere length [189]. Therefore, it is difficult to ascribe telomere shortening to oxidative stress or inflammation *in vivo*. The study of other oxidative/inflammatory compounds and longer exercise training regimes may help elucidate whether exercise attenuates telomere shortening via reduced ROS and inflammation.

Small non-coding RNA, such as miRNAs are ~22 bp in length and serve as negative regulators of translation by targeting the 3' untranslated region (UTR) on mRNA target transcripts or through mRNA degradation. While shelterin gene and protein expression have been studied in context with exercise training, little is known about the miRNAs regulating these. Data from our laboratory demonstrated decreased TERF2IP expression with increased miR-98 and miR-186 abundance after acute exercise training [178]. In breast cancer tissue miR-155 is up-regulated and down-regulates TRF1 protein abundance consequently promoting genomic instability [190]. Thus, the miRNA and other non-coding RNA, such as long non-coding RNA (including subtelomeric transcribed RNA, TERRA [191]), regulation of shelterin and telomere length changes associated with exercise is likely. Interestingly, miR-290-dependent regulation of retinoblastoma-like 2 protein which in turn impacts DNA methyltransferase (DNMT) enzyme activity and subsequent DNA methylation modulates telomere length and recombination [192]. To that end, telomere dynamics may also be influenced by epigenetic modifications and TERRA expression.

A change in gene expression without a change to the genetic sequence is the definition of epigenetic regulation. Epigenetic regulators such as DNA methylation and histone modifications are crucial for biological development and aberrant epigenetic profiles are associated with many age-related diseases [193, 194]. The impact of exercise training on genome-wide DNA methylation is beginning to be delineated and has been discussed in detail elsewhere – [195-198]. The *TERT* gene has a cytosine neighbouring a guanine dinucleotide (CpG) island (a region abundant in CpGs) located in its promoter region regulating telomerase expression in telomerase-positive cells [199]. There are, however, conflicting data on the effects of DNA methylation in the proximal promoter region of *TERT* in immortal and other cell lines [200]. It will be important to identify the influence methylation status has on TERT expression in leukocytes from healthy human donors. Furthermore,

482 the chromatin remodelling protein, CCCTC-binding factor, binds to exon 1 of *TERT* and represses *TERT* mRNA
483 expression, highlighting the complex epigenetic regulation of *TERT* [201]. DNA methylation in concert with
484 histone acetylation acts to negatively regulate *TERT* expression [202]. Work from the Blasco laboratory has
485 provided insight into the interactions between telomere length and epigenetic modifications [42, 44, 203].
486 Telomere shortening in *Terc*$^{-/-}$ mice is accompanied by histone methylation and acetylation changes at telomeric
487 and subtelomeric regions, consistent with a more open, transcriptionally active chromatin [42]. DNMT-null
488 mouse embryonic stem cells show exceptionally elongated telomeres, potentially due to telomere recombination
489 events [44]. Thus, there is ample evidence supporting a role for epigenetic regulation in the exercise-induced
490 changes to *TERT* and the expression of other telomere-associated genes, though this remains to be
491 experimentally demonstrated.

492 Therefore, it is likely that telomere length is tightly controlled by shelterin-mediated telomerase recruitment in
493 context with the cellular proliferative activity, which would undoubtedly be accelerated by increased oxidative
494 stress and inflammation. Considering inflammation and oxidative stress shorten telomeres *in vitro*, it is possible
495 that exercise may attenuate telomere attrition by maintaining a beneficial redox status and preventing chronic
496 low-grade inflammation. Whilst only beginning to be delineated, the role of epigenetic modifications, chromatin
497 structures and non-coding RNA, particularly miRNA and TERRA, molecules may serve as fine-tuners of
498 shelterin and telomerase activity. The aforementioned molecular and cellular factors could be responsible for the
499 telomere maintenance in individuals routinely engaging in aerobic exercise training or accelerated telomere
500 attrition observed in sedentary individuals (Figure 2).

501 **6 Conclusions**

502 The prevention of age-related cardio-metabolic diseases is an adaptation conferred by engagement in regular
503 exercise training, but the underlying molecular mechanisms are to date poorly understood. Cellular ageing
504 through telomere shortening is attenuated by physical activity and exercise training yet the most favourable
505 exercise prescription that maintains telomere length will require future study. The analysis of telomere length,
506 shelterin expression and telomerase activity with exercise training interventions involving different exercise
507 prescription including mode, frequency, duration and intensity, are warranted. Further, the impact of resistance
508 training on telomere length is relatively unknown and deserves attention. Efforts should be made to establish
509 additional molecular pathways by which exercise maintains telomere length. Analyses on oxidative stress,
510 inflammation, epigenetic modifications, non-coding RNAs (miRNAs and TERRA) and the telomere position

effect are recommended. These studies will help shed light on telomere-mediated healthy ageing and disease prevention through exercise training, to aid exercise prescription recommendations.

Compliance with ethical standards

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Conflicts of interest

Joshua Denham, Brendan O'Brien and Fadi Charchar declare that they have no conflicts of interest relevant to the content of this review.

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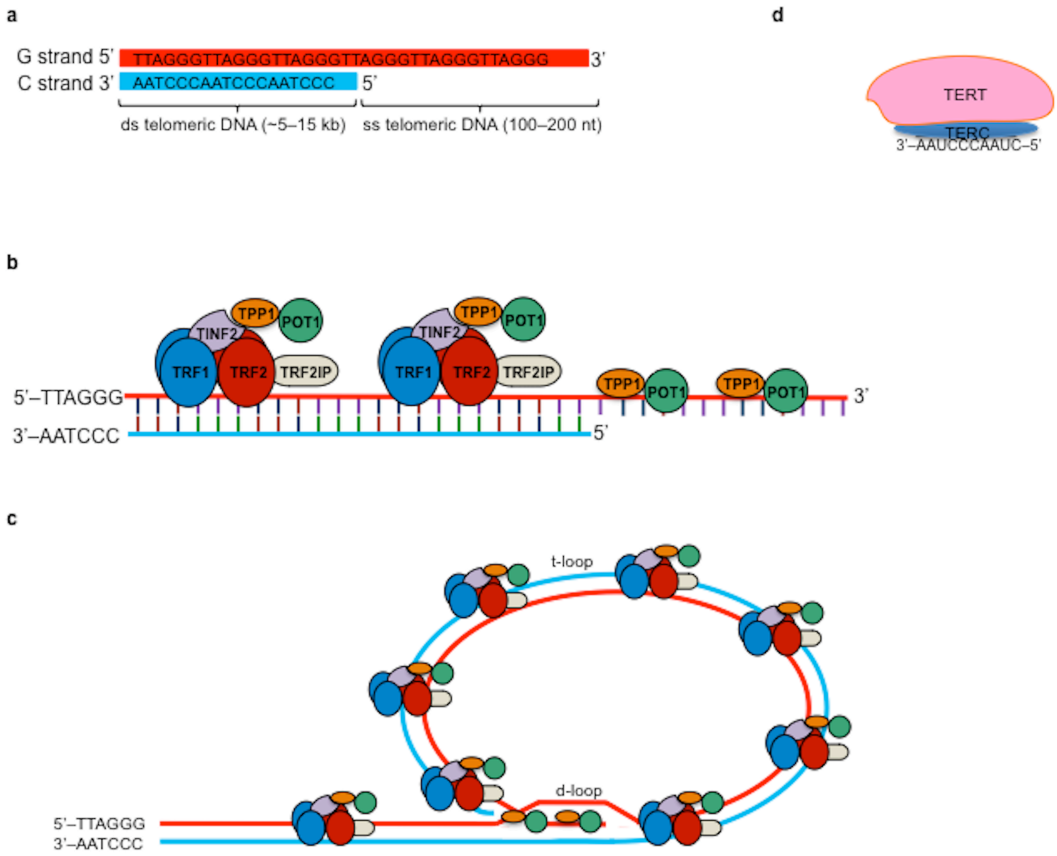
972

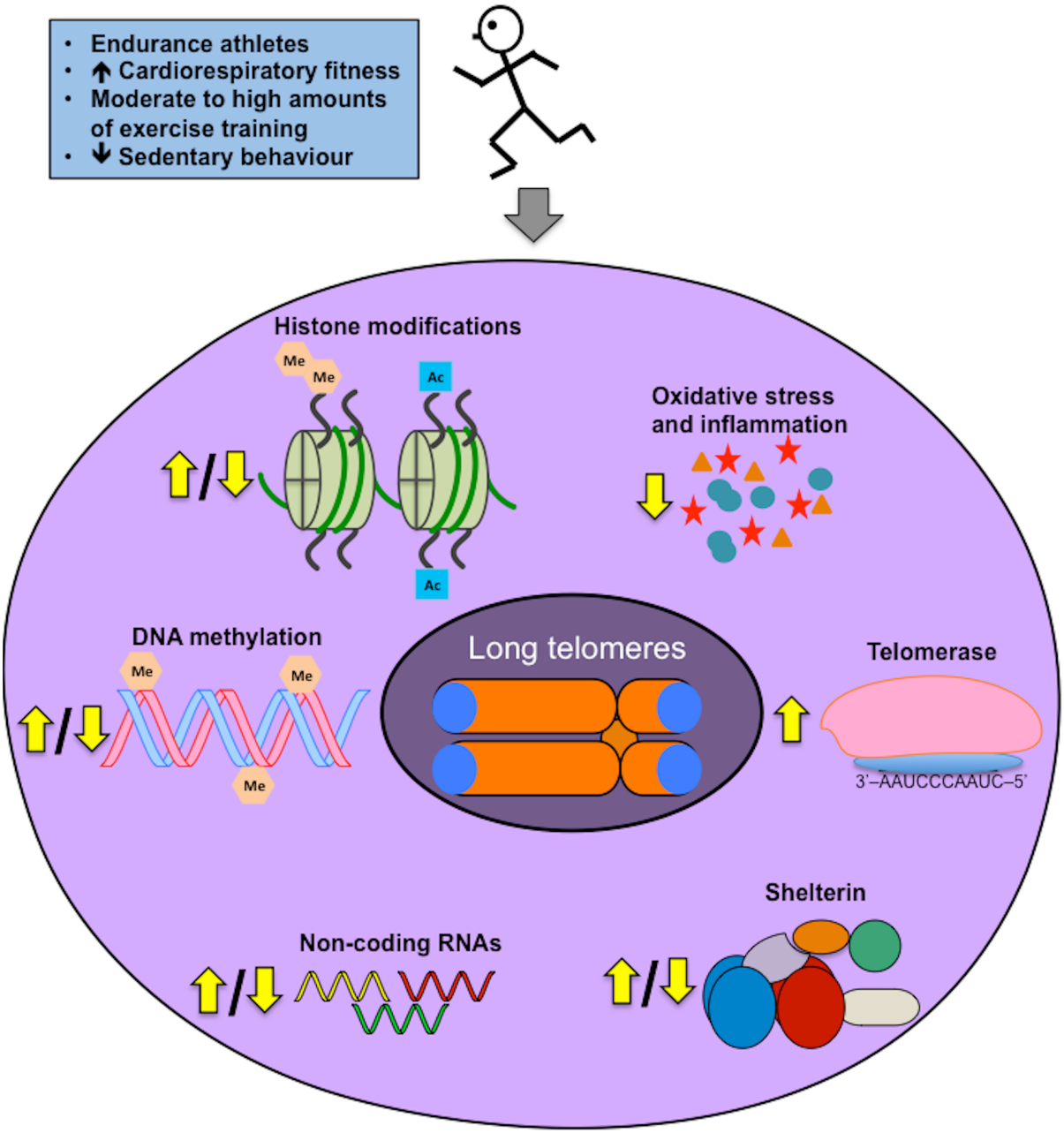
973 **Figures**



974

975 **Figure 1.** Telomeres, shelterin and telomerase. Telomeric DNA can form multiple structures that prevent
976 genomic recombination and fusion events. (a) Telomeres are comprised of the double (ds, leading strand) and
977 single-stranded (ss, lagging strand) DNA sequence – 5'-TTAGGG-3' and 3'-AATCCC-5', respectively. While
978 ds telomere DNA varies between 5 and 15 kilobases (kb) depending on age, disease status and lifestyle factors,
979 ss telomeric DNA is usually between 100 and 200 nucleotides (nt) long. (b) Ds and ss telomeric DNA are bound
980 by telomere-associated proteins collectively known as *shelterin*. TRF1 and TRF2 bind directly to ds telomeric
981 DNA. TINF2 and TPP1 bind POT1 to TRF1 and TRF2. TERF2IP binds to TRF2. TPP1 and POT1 also bind to
982 ss telomeric DNA. (c) Telomeres also form circular structures called t- and d-loops that prevent the abrupt
983 ending of telomeric DNA and DNA damage response pathways. (d) In humans, *telomerase* is formed by the
984 TERT protein and RNA molecule, TERC. The TERC RNA has a complementary sequence to telomeric DNA –
985 3'-AAUCCC-5' – and serves as a template for telomere elongation through a reverse transcription process.
986 TRF1, telomere repeat-binding factor 1; TRF2, telomere repeat-binding factor 2; TINF2, TRF1-interacting
987 nuclear factor 2; TPP1, adrenocortical dysplasia homolog; POT1, protection of telomeres 1; TERF2IP, TRF2-
988 interacting protein; TERC, telomerase RNA component; TERT, telomerase reverse transcriptase.

Figure 2. Mechanisms of telomere length maintenance conferred by exercise training.



Exercise training potential facilitates telomere length maintenance through many molecular mechanisms. Conversely, a sedentary lifestyle or lack of exercise training may deregulate the telomere maintenance pathways. Telomere length is likely regulated by epigenetic modifications including histone modifications (methylation [me] and acetylation [ac]) and DNA methylation. Non-coding RNAs such as miRNA may target shelterin mRNA to negatively regulate protein abundance and TERRA inhibits telomere elongation by binding to the TERC RNA template of telomerase competing against the telomeric DNA. Differential regulation of shelterin expression and telomerase activity would regulate telomere length. Finally, inflammation and oxidative

998 stress shortens telomeres *in vitro* and are candidates for telomere shortening *in vivo*. The yellow arrows indicate
999 whether the particular variable is increased or decreased, or possibly both – depending on the location or
1000 interaction with other factors – in context with exercise training. mRNA, messenger RNA; miRNA, microRNA,
1001 TERRA, telomeric repeat-containing RNA.

1002

1003 **Table 1** Current literature on exercise and telomere biology

Cell type	Participants[a,b,c,d,e]	Extraction method	Telomere measurement	Main result/s	Reference
	Athlete/control				
Leukocytes	61 endurance athletes and 61 healthy controls, M + F, ~30, VO_{2max} test and IPAQ.	Purelink Genomic DNA Mini Kit	qPCR	Endurance athletes exhibited longer leukocyte telomeres and increased *TERT* and *TPP1* mRNA expression compared to controls.	Denham et al. [151]
Buccal cells	20 endurance athletes and 42 sedentary controls, M + F, ~45.	QIAmp DNA Mini Kit	qPCR	Longer telomeres in athletes compared to controls.	Borghini et al. [204]
Leukocytes	392 former Finnish elite athletes and 207 controls, M, ~71, questionnaire.	?	qPCR	Former Finnish athletes have comparable age-adjusted telomere length compared to controls.	Laine et al. [153]
Leukocytes	67 ultra-marathon runners and 56 healthy controls, M, 43 ± 9.2.	?	qPCR	Ultra-marathon runners have 11% longer (16 years biologically younger) leukocyte telomeres compared to controls.	Denham et al. [150]

Lymphocytes and granulocytes	17 marathon runners and 15 sedentary controls, M + F, 54 ± 4, VO_{2max} test.	n/a	FISH	Similar telomere lengths between marathon runners and control. No statistically significant correlation between telomere length and VO_{2max}.	Mathur et al. [152]
Skeletal myocytes	5 young and older endurance athletes and healthy controls, M, young 24 and old 69, VO_{2max} test.	GenElute Mammalian Genomic DNA Miniprep Kit	qPCR	Older but not young endurance athletes have longer telomeres compared to their peers. Strong, positive correlation between VO_{2max} and telomere length.	Osthus et al. [156]
Skeletal myocytes	18 runners and19 sedentary controls, M + F, ~40.	Phenol/ chloroform	Southern Blot	Similar minimum and average telomere length between runners and sedentary controls. Moderate, inverse correlations between training history (years spent running and training hours) and minimum telomere length.	Rae et al. [160]

Leukocytes	27 (10 young and 17 older) endurance-trained individuals and 30 (15 young and older) sedentary controls, M + F, young 22 ± 1 and older 63 ± 2, VO_{2max} test.	?	Southern Blot	Older but not young, endurance-trained individuals had significantly longer leukocyte telomeres compared to their sedentary peers by ~900 bp. Moderate, positive correlation between VO_{2max} and telomere length.	LaRocca et al. [149]
Mononuclear cells Lymphocytes and granulocytes	57 (32 young and 25 older) endurance-trained individuals and 47 (26 young and 21 older) sedentary controls, M + F, young 21 and old 51, VO_{2max} test.	QIAmp DNA Blood Mini Kit	FISH and qPCR	Older sedentary individuals exhibited shorter mononuclear cell telomeres compared to all other groups. Up-regulated mononuclear cell telomerase activity in athletes (young and old) compared to sedentary controls.	Werner et al. [148]

Skeletal myocytes	7 power lifters and 7 sedentary controls.	Phenol/ chloroform	Southern Blot	A trend between higher minimum and average telomere length in power lifters compared to sedentary controls. Strong, inverse correlations between lower leg strength (squat and deadlift 1 repetition maximum) and minimum telomere length.	Kadi et al. [161]
Skeletal myocytes	13 FAMS and 13 healthy athletes, M + F, young, ~42 ± 11, VO_{2max} test.	Phenol/ chloroform	Southern Blot	Shorter minimum telomere length in FAMS compared to healthy athletes.	Collins et al. [159]

	Physical activity				
Leukocytes	6405, M + F, 20 – 84, questionnaire.	?	qPCR	Dose-dependent positive association between telomere length and physical activity levels.	Loprinzi et al. [205]
Leukocytes	582 older adults, M + F, 73 ± 5, modified Minnesota Leisure-Time Activities Questionnaire, timed 15-ft walk, chair stands and grip strength.	?	Southern Blot	Superior chair stand performance and greater walking distance associated with longer telomeres. Increasing physical activity and chair stand performance was associated with less telomere attrition in prospective analyses.	Soares-Miranda et al. [145]
Leukocytes	6405, M + F, 20 – 84, questionnaire.	?	qPCR	Every additional hour of screen-based sedentary behaviour is associated with 7% increased chance of possessing short telomeres.	Loprinzi [137]
Leukocytes	4576, M + F, ~55, binary.	Qiagen Blood Kit	qPCR	Long telomeres are associated with physical activity (at least 4 $hr \cdot week^{-1}$). Change in telomere length over 10 years is unaffected by physical activity.	Weischer et al. [206]

Leukocytes and skeletal myocytes	36 (12 young, 12 old-mobile and 12 old-immobile), M + F, young (25 y) and old (87.5 y).	QIAmp DNA Mini Kit	qPCR	Relative to old-immobile subjects, mobile older adults have attenuate oxidative stress and age-related telomere shortening in thigh muscle and leukocytes.	Venturelli et al. [158]
Leukocytes	239, F, 50-65, Stanford Brief Activity Scale.	QIAmp DNA Mini Kit	qPCR	Life-stress associated telomere attrition is attenuated by increased physical activity levels.	Puterman et al. [12]
PBMC	392, F, 62 ± 10, IPAQ.	?	Southern Blot	Postmenopausal breast-cancer patients who engage in moderate to high amounts of physical activity had longer telomeres than those not engaging in any physical activity by ~270 bp.	Garland et al. [136]
Leukocytes	204, M, 76, questions.	Gentra PureGene Blood Kit	Southern Blot	Compared to those engaging in low and high amounts of physical activity, those with self-reported moderate physical activity levels had the longest telomeres with less proportion of short telomeres.	Savela et al. [130]
Leukocytes	981, M + F, 45–84, Job Content Questionnaire	?	qPCR	No association with job-related physical activity.	Fujishiro et al. [134]

Leukocytes	5862 nurses, F, 58.7, questionnaire.	QIAmp 96 spin Blood Protocol	qPCR	Physical activity not associated with telomere length. Positive relationship between healthy lifestyle (non-smoking, moderate alcohol consumption, healthy body weight, moderate to high physical activity) and telomere length.	Sun et al. [207]
Leukocytes	44, F, 57.4 ± 5.6, card scanning.	G-spin Genomic DNA Extraction Kit	qPCR	Postmenopausal women regularly exercising (60 min, >3days/week) have longer telomeres than sedentary women.	Kim et al. [135]
Leukocytes	895, M + F, 33–79, three-point scale.	QIAmp	qPCR	Exercise frequency is an independent predictor of leukocyte telomere length . A healthy lifestyle explained 40% of the association between intelligence and leukocyte telomere length.	Kingma et al. [208]
Leukocytes	7813 nurses, F, 43 - 70, questionnaire.	QIAmp 96-Spin Protocol	qPCR	Longer telomeres in nurses who engaged in moderate or high amounts of physical activity, and moderate to vigorous intensity activities.	Du et al. [128]
Leukocytes	667 healthy adolescents, M + F, 14 – 18, accelerometer.	?	qPCR	Positive association between vigorous physical activity and telomere length in girls.	Zhu et al. [146]

Leukocytes	2284 nurses, F, 30 – 55, questionnaire.	QIAmp 96 DNA Blood Kit	qPCR	No association between physical activity and telomere length.	Cassidy et al. [132]
Leukocytes	63 healthy postmenopausal women, F, 62, questions.	Puregene DNA Isolation System	qPCR	Regular physical activity ameliorates the negative relationship between psychological stress and telomere length.	Puterman et al. [165]
Leukocytes	82, M + F, 18 – 80, questions.	Puregene Blood Core Kit	qPCR	No correlation between exercise and telomere length.	Song et al. [131]
Leukocytes	318 healthy individuals, M + F, 51.3, questions.	Gentra Puregene Blood Kit	qPCR	No interaction between telomere length and exercise and the presence of coronary artery calcium.	Diaz et al. [133]
Leukocytes	612 advanced prostate cancer patients and 1049 controls, M, 55 – 74, ?	QIAmp 96 DNA Blood Kit	qPCR	No correlation between physical activity and telomere length. Positive correlation between a healthier lifestyle (physical activity, diet, low BMI and low or no cigarette smoking) and telomere length.	Mirabello et al. [209]

Leukocytes	2006, M + F, 72 ± 5, PASE.	?	qPCR	No association between physical activity and telomere length.	Woo et al. [210]
PBMC	69, M + F, 60 ± 4.9, YPAS.	PureGene DNA Isolation System	qPCR	Moderate estimated energy expenditure associated with longest telomeres compared to low and high energy expenditure.	Ludlow et al. [129]
Skeletal myocytes	16 young and 26 older recreationally-active individuals, M + F, young 25 ± 4 and old 74 ± 4.	Phenol/ chloroform	Southern Blot	Similar minimum and average telomere lengths between young and older recreationally-active individuals.	Ponsot et al. [157]
Leukocytes	2401 white twins, M + F, 18 – 81, Allied Dunbar National Fitness Survey.	?	Southern Blot	Strong, positive relationship between physical activity and telomere length. More active twin had longer (~88bp) telomeres. Most active individuals had longer (~200bp) telomeres than their inactive peers.	Cherkas et al. [127]

	Physical fitness				
Leukocytes	1764, M + F, 20 – 49, submaximal treadmill test.	?	qPCR	Compared to individuals with poor predicted cardiorespiratory fitness (30.7 $ml \cdot kg^{-1} \cdot min^{-1}$), those with moderate (39.1 $ml \cdot kg^{-1} \cdot min^{-1}$) or high fitness (50.9 $ml \cdot kg^{-1} \cdot min^{-1}$) possessed longer leukocyte telomeres.	Loprinzi [211]
PBMC	2488, M, middle age, vertical jump test, handgrip test, sit to stand and spirometry test.	?	qPCR Universal STELA	Number of short telomeres (<750 bp) are inversely associated with maximum jump height.	Maynard et al. [212]
Leukocytes	277 individuals, M + F, 76, grip strength.	QIAmp DNA Maxi Kit	qPCR	Faster telomere attrition associated with the decrease in grip strength after a 10-year follow-up.	Baylis et al. [144]
Leukocytes	117 elderly Koreans, F, 42 ± 0.7.	G-spin Genomic DNA Extraction Kit	qPCR	Medium, positive correlation between gait speed (6m) and telomere length.	Lee et al. [140]

Leukocytes	548 same-sex twins, M + F, 78.3, Physical Ability Score and five-point scale.	?	Southern Blot	Positive associations between physical ability score, physical activity and leukocyte telomere length.	Bendix et al. [143]
Leukocytes	44 Japanese patients, M + F, 74, physical assessment.	?	Southern Blot	Physical capacity associated with greater proportion of long telomeres (>9400bp) in females.	Maeda et al. [141]
Leukocytes	944 coronary heart disease outpatients, M + F, ~67, questions.	?	qPCR	Linear relationship between exercise capacity (maximum METs obtained during treadmill testing) and telomere length. Self-reported physically active individuals have longer telomeres (~73bp) than their inactive peers.	Krauss et al. [155]
Leukocytes	23 Japanese cerebrovascular disease patients, F, 77.7 ± 6.4, physical assessment.	?	Southern Blot	Physical capacity associated with greater proportion of long telomeres (>9400bp).	Maeda et al. [142]

A single bout of exercise					
Buccal cells	20 endurance athletes and 42 sedentary controls, M + F, ~45	QIAmp DNA Mini Kit	qPCR	Relative to basal, athletes had significantly shorter telomeres mid-way and at completion of an ultra-marathon trail race.	Borghini et al. [204]
Leukocytes T lymphocytes	22, M, 24 ± 7.	n/a	n/a	Acute, high-intensity exercise modulates leukocyte *TERT*, *SIRT6*, *TERF2IP* mRNA and microRNA (miR-15a, -96, -181 and -186) abundance.	Chilton et al. [178]
PBMC Skeletal myocytes	8, M + F, 44 ± 2.	Promega Wizard SV Kit	qPCR	Telomere-associated gene mRNA was increased in PBMC (*TERF1*, *TERF2*, *POT1*, *KU70* and *KU80*) and skeletal (*KU70* and *KU80*), without changes to protein content after a seven-day ultra-marathon race. No change to *TERC* and *TERT* mRNA abundance, telomere length or telomerase activity.	Laye et al. [179]
T lymphocytes	9 moderately trained (VO_{2max}: 56.9 ± 5.1), M, 26 ± 6.7.	?	qPCR	Acute, high-intensity exercise increases CD8+ T-cell telomere length, but does not alter CD3+ or CD4+ T-cell telomere length.	Simpson et al. [213]

PBMC	10 young (median age: 23) and 10 older (median age: 78) physically active individuals, M + F.	Qiagen Blood Kit	Southern Blot	Average telomere length decreases after acute exercise in young (PBMC and CD8+) and older (CD4+) individuals.	Bruunsgaard et al. [214]

	Training studies				
PBMC	59, M, 45–65, VO_{2max} test, ?	?	Southern Blot	Longer telomere and elevated telomerase activity after six months of exercise training.	Melk et al. [164]
Leukocytes	49 sedentary and overweight older adults, M + F, 68 y, IPAQ, 6-month intervention involving counseling and prescribed exercise.	?	qPCR	Reduced sitting time correlated to telomere lengthening.	Sjögren et al. [138]
PBMC	10 cases and 25 controls with prostate cancer, M, ~62.5, n/a, walking 30 min 6 days/week for 3 months (healthy diet, stress management, meditation and counseling).	?	qPCR	Longer telomeres after a 5-year lifestyle intervention.	Ornish et al. [163]

Leukocytes	439 overweight/obese, F, 58, VO_{2max} test, 45 min of moderate to vigorous (70-80% HR_{max}) exercise 5 days/week for 1 year (with or without a calorie-reduced diet).	Qiagen Midi Kit	qPCR	Weak, positive correlation between telomere length and VO_{2max}. No change to telomere length after 12-month intervention.	Mason et al. [154]
Leukocytes	190 cases and 188 controls, M + F, ~55, n/a, increase physical activity.	Salting out method	qPCR	Lifestyle intervention did not have an effect on telomere length after a 4.5 y follow-up.	Hovatta et al. [215]

PBMC	24 prostate cancer patients, M, 62.2 ± 7.5, n/a, walking 30 min 6 days/week for 3 months (healthy diet, stress management, meditation and counseling).	n/a	n/a	Increased telomerase activity after lifestyle intervention.	Ornish et al. [162]
Leukocytes	16 obese individuals, F, 46.8 ± 6.4, VO_{2max} test, 60 min of treadmill walking/running @ 60% VO_2 reserve thrice weekly for 3 months.	Wizard Genomic DNA Kit	qPCR and Southern Blot	No change to telomere length after exercise moderate intensity exercise training.	Shin et al. [189]

	Rodent studies				
Hippocampus	45 Sprague-Dawley rats, M, pups, n/a, voluntary wheel running.	AllPrep DNA/RNA Mini Kit	qPCR	Voluntary wheel running prevented telomere elongation associated with maternal separation – a model of developmental stress.	Botha et al. [216]
Skeletal myocytes	22 C57BI/6 mice, F, 6 weeks old, n/a, 30 min of treadmill running @ 65% of V_{max}.	n/a	n/a	Acute exercise decreases *Trf1* expression in conjunction with increased p38-MAPK phosphorylation. No changes to other shelterin gene expression.	Ludlow et al. [180]
Liver, skeletal and cardiac myocytes	CAST/Ei J mice (6 young 8-week old, 11 1 year old sedentary and 10 1 year old exercised), M + F, 8 weeks and 1 year old, voluntary wheel running.	PureGene DNA Isolation System	qPCR	Voluntary wheel running attenuates cardiac myocyte and hepatocyte telomere length attrition, but accelerates skeletal myocyte telomere shortening. Up-regulated telomerase activity in skeletal myocytes after voluntary wheel running. Voluntary wheel running attenuated the age-related reduction in expression of telomere-associated genes (*Trf1*, *Trf2* and *Pot1*) in skeletal and cardiac myocytes but not hepatocytes.	Ludlow et al. [175]

Hippocampus	4 nestin-green fluorescent protein (expressing) and 12 C57BI/6, ?, n/a, 10 days of voluntary wheel running.	n/a	FISH	Voluntary wheel running restored lowered and further increased telomerase activity in a mouse-model of schizophrenia and controls, respectively. No change to telomere length after voluntary wheel running.	Wolf et al. [177]
Aortic and spleen-derived mononuclear cells	C57BI/6 wild-type and *Tert*$^{-/-}$ and *eNos*$^{-/-}$ mice, ?, n/a, n/a, voluntary wheel running for either 3 weeks or 6 months.	n/a	FISH	Increased telomerase activity, Trf1 and Trf2 expression, with decreased pro-apoptotic proteins (Chk2, p16 and p53) after 3 weeks of voluntary running in wild-type, but not *Tert*$^{-/-}$ and *eNos*$^{-/-}$ mice. No change to aortic telomere length after voluntary running.	Werner et al. [148]
Cardiac myocytes and leukocytes	C57Bl/6 wild-type and *Tert*$^{-/-}$ and *eNos*$^{-/-}$ mice,?, n/a, n/a, voluntary wheel running for either 3 weeks or 6 months.	n/a	FISH	While Tert and Trf2 protein expression and telomerase activity were up-regulated, pro-apoptotic proteins (Chk2, p16 and p53) were ameliorated after 3 weeks and 6 months of voluntary running in wild-type, but not *Tert*$^{-/-}$ and *eNos*$^{-/-}$ mice. Left ventricular myocyte and leukocyte telomere length unchanged after voluntary running.	Werner et al. [176]

[a] number and type of participants; [b] sex; [c] average age ± standard deviation or range; [d] method of assessing physical activity/estimated energy expenditure/exercise/fitness; [e] exercise training (other lifestyle interventions).

M = male; F = female; n/a = not applicable; qPCR = quantitative polymerase chain reaction; FISH = fluorescence in situ hybridization; STELA = single telomere length analysis; VO_{2max} = maximal oxygen consumption; bp = base-pairs; PBMC = peripheral blood mononuclear cell; TERT = Telomerase reverse transcriptase; TPP1 = adrenocortical dysplasia homolog; SIRT6 = Sirtuin-6; TERF2IP = Telomere repeat binding factor 2 interacting protein; IPAQ = International Physical Activity Questionnaire; PASE = Physical Activity Scale for the Elderly; YPAS = Yale Physical Activity Survey; HR_{max} = heart rate maximum; $^{-/-}$ = deficient (gene); FAMS = fatigued-athlete myopathic syndrome; Vmax = maximal treadmill speed; eNos, endothelial nitric oxide synthase; BMI = body mass index; MET = metabolic equivalent of task; MAPK = mitogen-activated protein kinase; Pot1 = protection of telomeres-1; Trf = telomere-repeat binding factor; ? = unknown.